

THE GEORGIAN BAY SURVEY

BACTERIOLOGY REPORT

July, 1970

by

Moya Jones

Bacteriology Branch

Division of Laboratories
ONTARIO WATER RESOURCES COMMISSION

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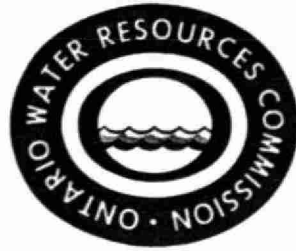
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ABSTRACT

The bacteriological water quality in Penetang, Midland and Hog Bay was evaluated by determining the coliform, fecal coliform, fecal streptococci, total heterotroph and Clostridium densities. The areas were sampled from May to October on a biweekly basis.

The Water Pollution Control Plant in Penetang was found to be largely responsible for the pollution present in the South Basin. Midland and Hog Bay stations were much cleaner, and stations P3 and P4 in Penetang Bay appeared as yet unaffected by pollution.

Colonies from heterotrophic count plates were isolated and identified. Changes in the generic composition of the bacterial flora occurred with changes in coliform and total heterotroph densities, and with temperature.

The Acinetobacter and fluorescent pseudomonads have been implicated as possible indicators of eutrophication. For this reason, experimental enumeration methods for these two organisms were investigated. The results indicated that the media were reliable enough to warrant further testing.

Introduction

This study was undertaken to obtain an evaluation of the bacteriological water quality in Penetang, Midland and Hog Bay, and to determine the major sources of pollution and the extent of their effect in these areas. This was accomplished by means of membrane filtration analyses for the pollution indicator organisms. In addition, information on the types and distribution of heterotrophic bacteria was collected by means of isolation and identification of colonies from total heterotrophic count plates. An attempt was made to correlate microbial populations in this area with the findings reported by Bennett (1969).

In 1964, the Division of Sanitary Engineering carried out a survey of Penetang Bay. At that time the Water Pollution Control Plant had not yet been installed. The entire bay was sampled (about 90 samples), but approximately half of the total samples were taken on the east side of the South Basin (near the Town of Penetang). Between 25.0 and 38.0% of the samples demonstrated coliform densities greater than 1000 organisms per 100 mls. These unacceptable counts were all from sample points within the South Basin.

In 1967, after the installation of the Water Pollution Control Plant, the survey was repeated by the Division of

Sanitary Engineering. During this survey a total of 30 samples were taken throughout the bay and all samples contained less than 1000 coliforms per 100 mls.

Midland Bay was surveyed by the Division of Sanitary Engineering in 1966. Counts from samples from storm sewage outfalls and the Water Pollution Control Plant outfall were higher than the accepted level of 1000 coliforms per 100 mls. Samples from the body of Midland Bay itself however demonstrated acceptable counts.

In addition to the bacteriological studies outlined herein, this survey also involved the determination and investigation of biological and chemical characteristics of the area. A description of these aspects of the survey may be found in the OWRC report entitled: "A Report on Water Quality in the Penetang - Midland - Port McNicoll - Waubaushene Area of Georgian Bay". (D.M. Veal, M.F.P. Michalski, A.R. Clark, and M. Jones, 1970).

METHODS

1. Field Procedures:

A description of the survey area and general field procedures was presented in the main report (D.M. Veal et al., 1970). Apart from the stations described therein, two additional stations were sampled for bacteriological analyses. These stations were located at the effluent outflows of the Penetang and Midland Sewage Treatment Plants. The outflow in Penetang was approximately 20 feet from shore directly in line with the plant. In Midland, the discharge at the foot of William Street was sampled approximately 10 feet from shore in line with the outlet. Bacteriological samples were not taken from stations BS and PM1.

In addition to the eleven regular biweekly sampling times, an intensive survey, during which consecutive daily samples were taken, was conducted from July 14 to July 24. Samples from this survey were subjected to the same analyses as the regular biweekly samples.

Approximately 250 mls. volumes of water were collected for bacteriological analyses, and these samples were iced immediately and stored this way until analyzed. Membrane filtration analyses were carried out by the Great Lakes Field Staff at the Toronto laboratory.

2. Laboratory Procedures:

All analyses were performed within the 24 hours following sampling. Total plate count analyses were accomplished within 12 hours. Enumeration by membrane filtration included counts for total coliforms, fecal coliforms, fecal streptococci, Clostridia and total heterotrophs (total plate count). Counts were recorded as the number of organisms per 100 mls.

Coliform analyses were conducted according to Standard Methods (1965), using m-Endo Broth (Difco). MacConkey Membrane Broth (Oxoid) was used to cultivate fecal coliforms, with incubation at 44.5°C in a water bath for 18-20 hours. For fecal streptococci determinations, m-Enterococcus Agar (Difco) was used, with incubation at 35°C for 48 hours. The culture medium for total plate counts was m-Plate Count Broth (Difco) buffered with 0.1% K_2HPO_4 . Incubation was at 20°C in a humid atmosphere for 72 hours. The use of black membrane filters (Millipore) facilitated counting of colonies. Clostridia were cultivated on SPS Agar (BBL). Incubation was at 45°C for 28 hours in an anaerobic jar with a carbon-dioxide, hydrogen generating Gaspak (BBL). Inversion and immersion of the membrane filter into the still molten agar produced more clearly defined colonies.

Colonies growing on total plate count plates were subjected to isolation and identification procedures. From membrane filters containing between 20 and 200 colonies, ten colonies were picked from one sector of the plate. The isolates were streaked on Trypticase-Soy (T-Soy) Agar and incubated at 20°C for five days. Following incubation, colonial morphology was described and single colonies were streaked on T-Soy Agar slants. The slants were incubated at 20°C for 48 hours at which time Gram stains (Kopeloff-Beerman modification, Manual of Microbiological Methods, 1957) were done to check culture purity and to allow description of microscopic morphology.

Pure cultures thus obtained were then subjected to a battery of diagnostic tests. These included tests for the presence of catalase and oxidase (Kovacs, 1956), motility (hanging drop method with two passages through T-Soy Broth), acid and gas production in Phenol-Red Dextrose Broth, reaction in the glucose Oxidation-Fermentation Medium of Hugh & Leifson (1953), and growth on MacConkey Agar. These tests, referred to as primary identification tests were carried out at 20°C. The cultures were identified on the basis of these tests according to schemes presented by Cowan & Steel (1965), as outlined by Bennett (1969). Spore stains (Shaeffer and Fulton's Method, Cowan & Steel, 1965) were

performed on Gram-positive bacteria and those Gram-negative organisms which failed to grow on MacConkey Agar.

Those cultures, identified as members of the Enterobacteriaceae, were further tested for the fermentation of lactose, the decarboxylation of lysine and ornithine, the dihydrolase of arginine, the production of indole, the methyl-red and Voges-Proskauer reaction, the utilization of citrate, the presence of urease and phenylalanine deaminase, the triple-sugar iron agar reaction, and gelatin hydrolysis. These secondary identification tests were carried out at 37°C. The classification of Enterobacteriaceae cultures was based upon these tests plus motility and glucose fermentation repeated at 37°C, according to the scheme presented by Edwards and Ewing (1962), and amended on the basis of the findings of the Enterobacteriaceae Subcommittee of the Nomenclature Committee of the International Association of Microbiological Societies (Enterobacteriaceae Subcommittee, 1958, Ewing and Edwards, 1960; Carpenter, 1963, Ewing, 1963).

Members of the Aeromonas group were tested for the presence of lysine and ornithine decarboxylase, and arginine dihydrolase. These tests were incubated at 20°C.

A detailed description of reagents, media and test procedures was presented in Appendix I of Bennett's report (1969).

Two experimental membrane filtration enumerations were carried out. The first was for Acinetobacter using the media described by Mandell (1964). Plates were incubated at 20°C for 48 hours. Colonies of Acinetobacter were a pale lavender colour, similar to the colour of the medium. A number of characteristic colonies were picked from these plates and subjected to the same identification tests as those for total plate count isolates.

The second experimental media was for fluorescent pseudomonads. Drake's basal medium (Drake, 1966) was used to cultivate these organisms. Incubation was at 20°C for 48 hours. Fluorescent colonies were counted in the dark using an ultra-violet light source. A limited number of these colonies were picked and subjected to identification tests.

3. Statistical Methods:

The formulae used for calculated statistics were as follows:

(i) Geometric mean: $G.m. = \text{antilog } \frac{\sum x}{N}$

(ii) Standard deviation: $S = \sqrt{\frac{\sum (x - \bar{x})^2}{N-1}}$

$$\bar{x} = \frac{\sum x}{N}$$

(iii) 95% confidence limits of the mean: $\log G.m. \pm t \frac{S}{\sqrt{N}}$

where N equals the number of observations, x is the common logarithm of the individual value, and t is the critical value of the Student's t distribution at the 0.05 level of confidence for N-1 degrees of freedom.

$$x^2 = \frac{\sum (x - \bar{x})^2}{\bar{x}}$$

The degrees of freedom for the critical x^2 value was N-1.

The critical values for the Student's t and chi-squared distributions were obtained from tables by Rohlf and Sokal (1969).

RESULTS AND DISCUSSION

1. Membrane Filtration Analyses:

The data were grouped according to station and counts were converted to common logarithms. For each station the geometric mean and the 95% limits of the mean were calculated on the basis of the eleven biweekly sampling times. These statistics were illustrated graphically in Figures 1 and 2.

The distances from Sewage Treatment Plant effluents to sampling points within Penetang and Midland Bays were estimated from Canadian Hydrographic Services maps (nos. 2216, 2217). In Figures 3 and 4, the log geometric means were plotted as a function of this distance.

From Figures 3 and 4, it was clear that an increase in distance from effluent outflows induced a decrease in counts. This trend in Penetang Bay produced significantly lower mean counts, demonstrated by the mean confidence limits in Figure 1. In Midland Bay, the differences between mean counts were not as appreciable, although a decreasing trend was still evident.

The Sewage Treatment Plant in Penetang Bay appeared to be a source of bacterial contamination. The plant effluent however was disinfected by chlorination. It was first suggested that the high population counts recorded at

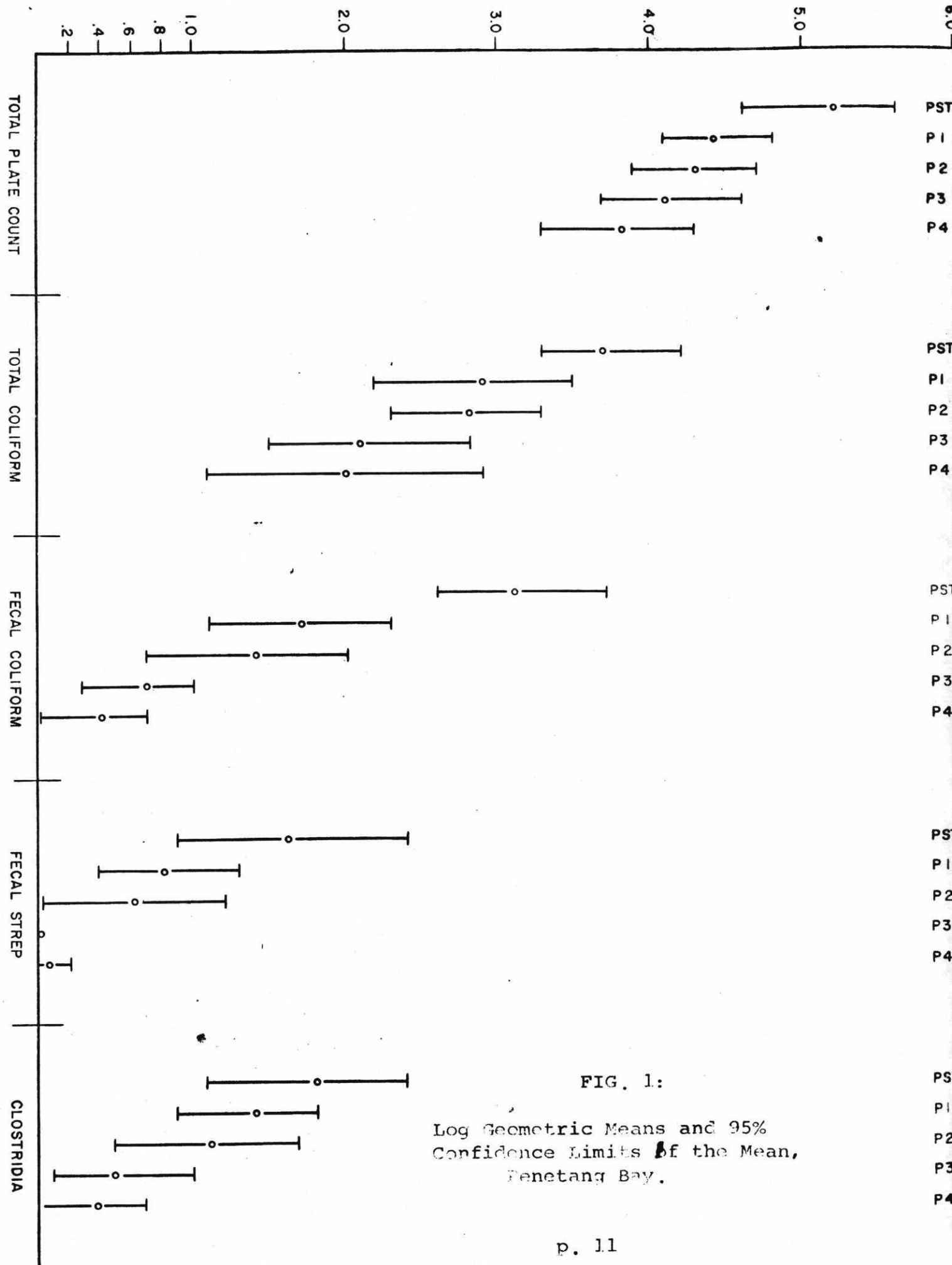


FIG. 1:
Log Geometric Means and 95%
Confidence Limits of the Mean,
Penetang Bay.

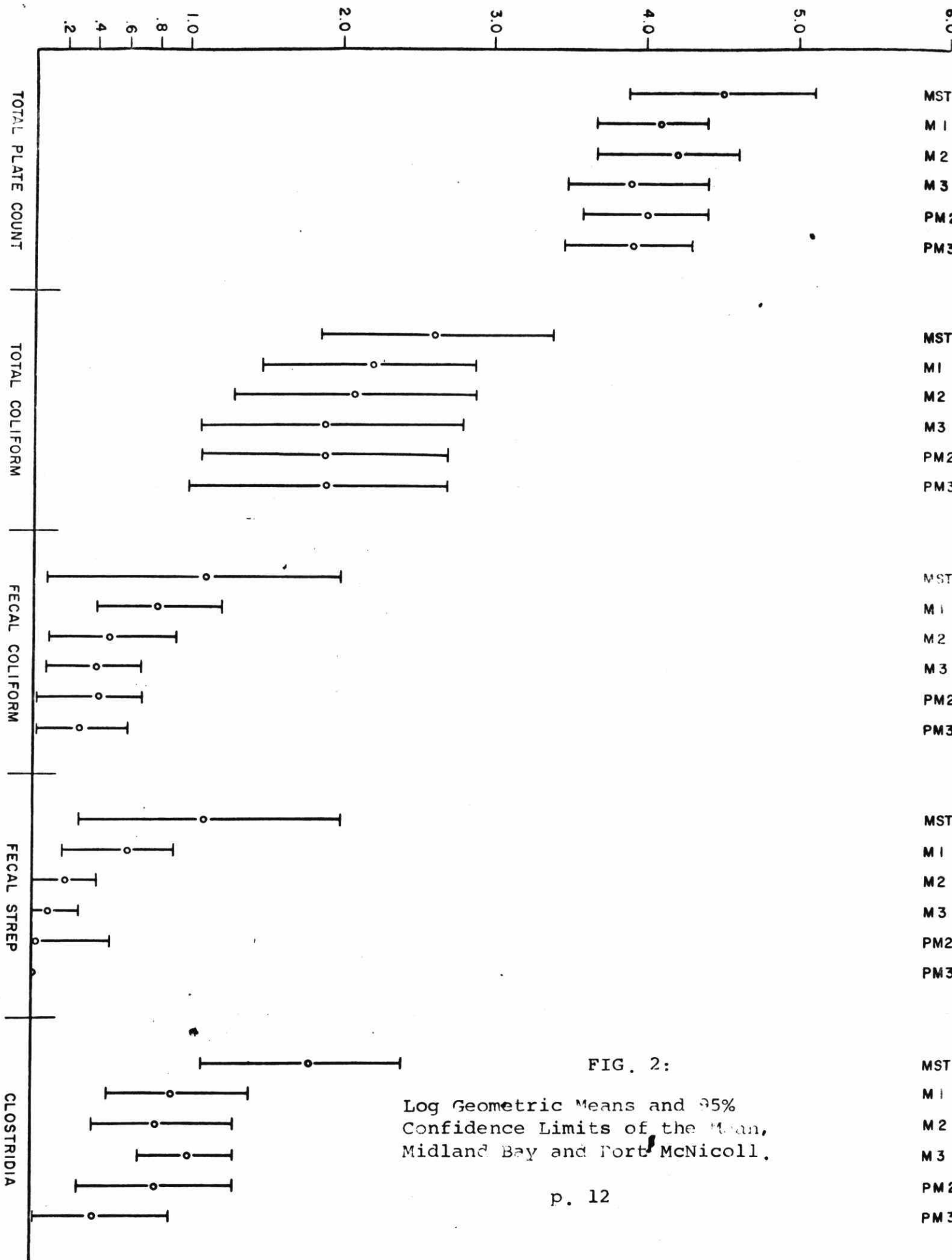


FIG. 3: Log Geometric Means versus Distance,
Penetang Bay.

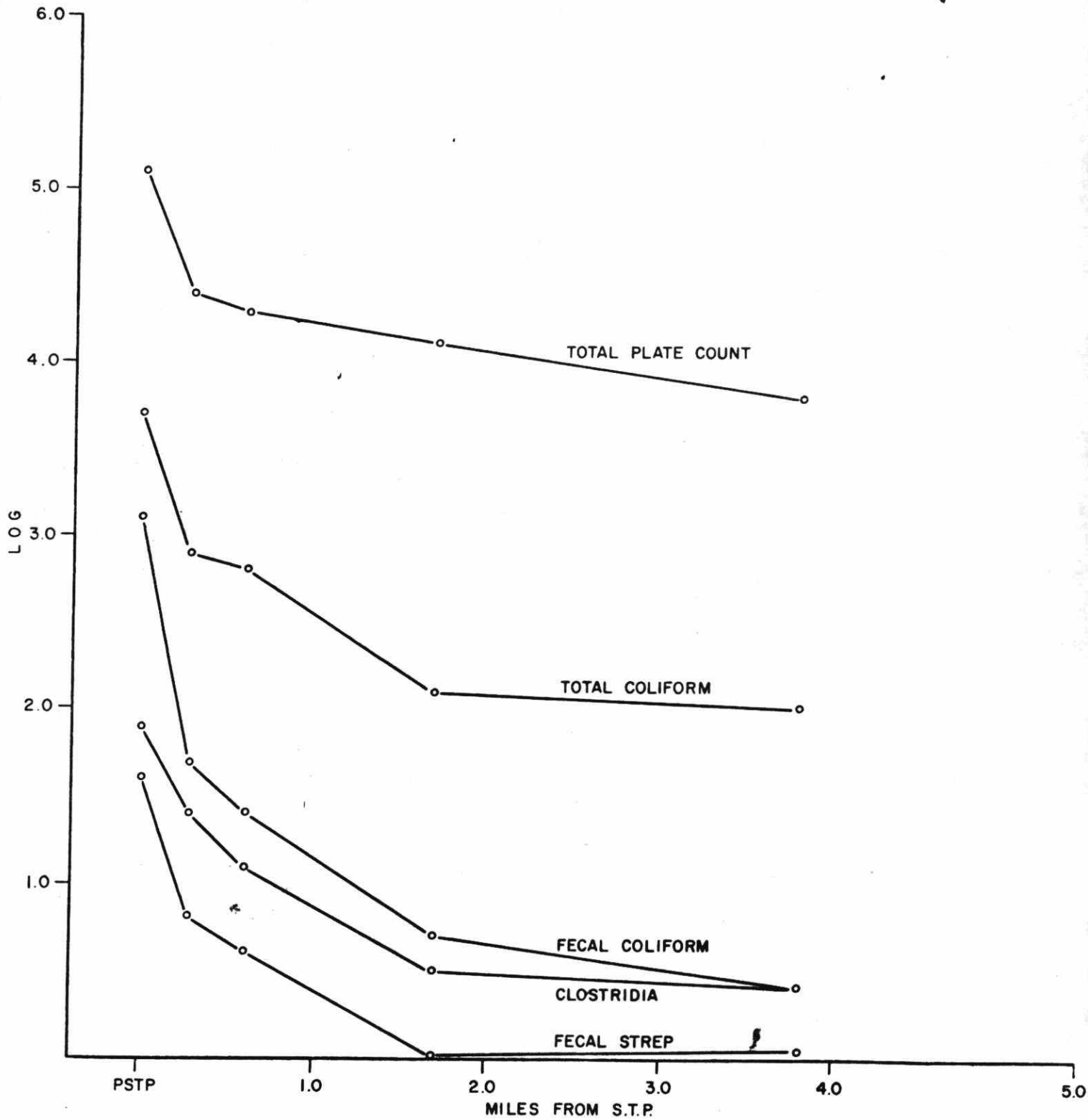
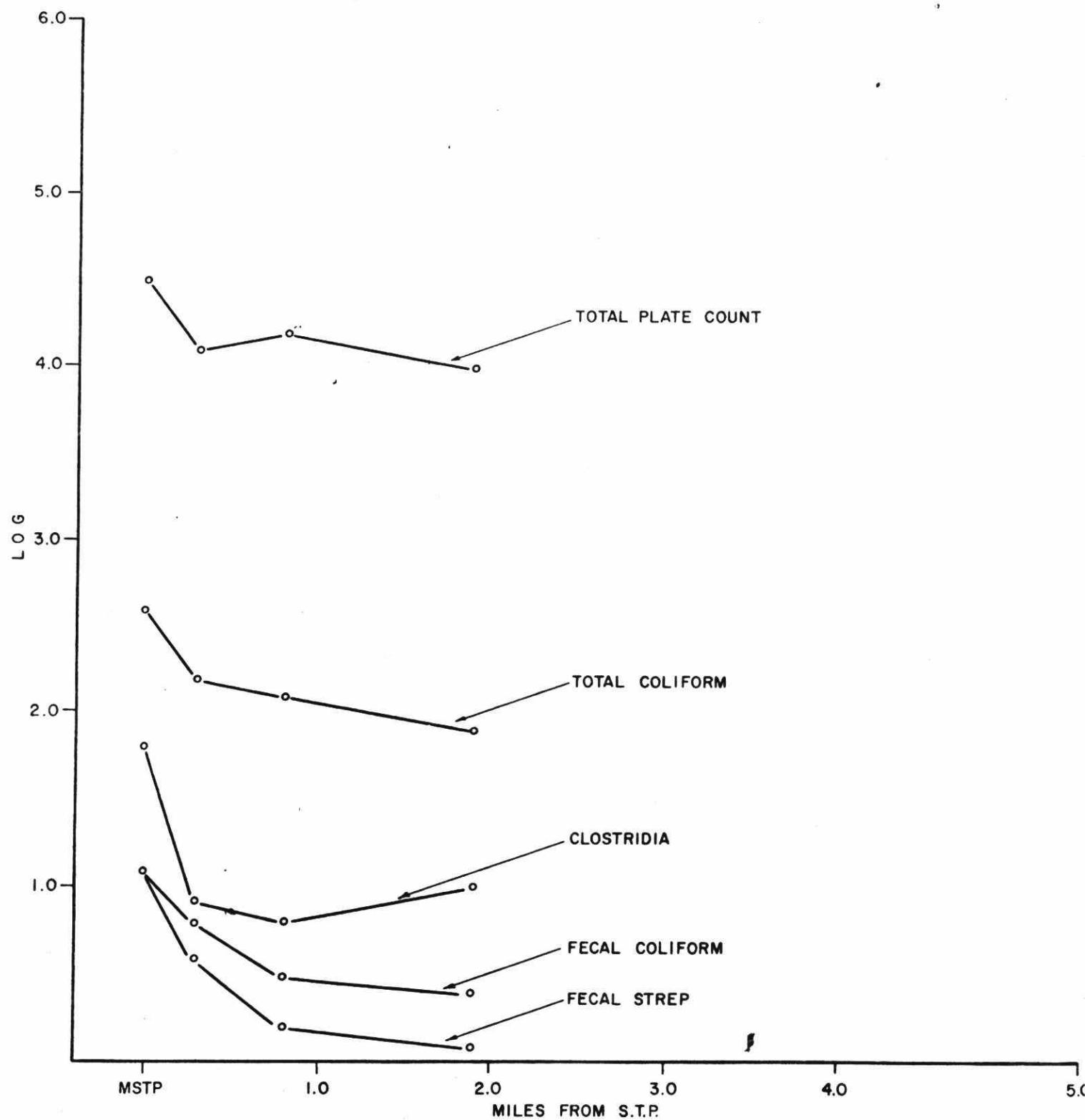


FIG. 4: Log Geometric Means versus Distance,
Midland Bay.



PSTP were due to nutrient stimulation of bacteria already present in the water. This fact could not explain the high levels of fecal coliform and fecal streptococci present. These organisms, particularly the latter, are considered incapable of proliferation within the aquatic environment due to their requirement for a large variety of amino acids and vitamins. It was at this point that bypass procedures within the plant were considered as possible causes of such elevated counts.

Data concerning days on which bypass occurred was not available for the months of May through August. During September and October however, occasions of bypass and sampling coincided. This occurrence allowed some estimation of the effects of bypass.

On September 23, bypass occurred at the Penetang Sewage Treatment Plant. The duration of bypass and volume passed was not known. Total plate count (TPC), coliform (TC), fecal coliform (FC) and fecal streptococci (FS) counts were elevated at this time. (TPC: 490,000, TC: >10,000, FC: 830, FS: 98 organisms per 100 mls.) There was no apparent effect on Clostridia.

Another bypass took place on October 21, and continued for fourteen hours. On this occasion, all counts, including

the Clostridia (CL), were very high. (TPC: 440,000, TC: 32,000, FC: 6,200, FS: 280, CL: 220 organisms per 100 mls.) At the time of sampling, elevated counts were also obtained from stations P1 and P2 (approximately a third and two thirds of a mile away, respectively).

An interesting situation was reflected by counts from samples taken on October 8. The previous day, bypass had occurred for eleven hours. At station PSTP, the highest counts of the survey were recorded for total plate count, fecal coliform and fecal streptococci counts. (TPC: 3,040,000, FC: >10,000, FS: 704 organisms per 100 mls.) The coliform count was extremely low (480 organisms per 100 mls.), of less magnitude in fact than either the fecal coliform or fecal streptococci counts. The effect of the previous day's bypass was also reflected by counts at station P1, when total plate count, fecal coliform and fecal streptococci were high. The coliform count demonstrated a depression similar to that which occurred at station PSTP. At station P2, only total plate count, which was elevated, seemed affected by the bypass.

It is difficult to explain the high counts of fecal coliform and fecal streptococci obtained on October 8. These counts, recorded 24 hours after a bypass, were higher than those actually recorded during a bypass. This fact would

seem to imply not only survival of these organisms within the aquatic environment, but a multiplication of the introduced populations.

That coliform counts were depressed at the same time that fecal coliform and fecal streptococci were elevated, was unexpected. This situation would seem to indicate a system of selective inhibition. It is difficult to envisage any chemical inhibitor capable of so adversely affecting coliforms yet not interfering with the more exacting organisms such as fecal streptococci. The presence of a coliform-phage was suggested. The naturally high levels of the coliform population would allow such a phage to establish itself as a permanent member of the environment. Assuming this phage could convert from temperate to virulent phases, such a conversion would explain the sudden and drastic decrease in coliform counts. The occurrence of such a mass lysis could conceivably provide the amino acids and vitamins required by fecal coliforms and fecal streptococci for proliferation.

At stations P2, P3 and P4, coliform counts tended to reflect more readily the changes of temperature over the course of the sampling period. Temperatures, when the survey started, were in the range of 8 - 10°C. During

July they reached a peak of 23 - 25°C and had declined again to 8 - 10°C by October. Coliform counts at the above stations showed, if not a parallel course, a similar rise and fall pattern. At station PSTP, counts remained consistently high throughout, and at station P1, large fluctuations occurred independent of temperature.

Midland Bay, in comparison with Penetang Bay, was much cleaner. The geometric means were high at station MSTP (Fig. 2). Counts from this station however were not nearly as high as those recorded at PSTP (e.g. August 11, TC: 7,000 and 760 per 100 mls. for PSTP and MSTP, respectively), and in most cases were lower than those recorded at station P1 (August 11, TC: 2,600 per 100 mls.). Due to these lower counts at MSTP, differences between mean counts of Midland Bay stations were not as significant statistically as those seen in Penetang Bay. A decreasing trend was still evident however with an increasing distance from the Sewage Treatment Plant.

The Wye River was also a contributing factor to the level of counts in Midland Bay. A reflection of its effect was expected in counts from station M2. Counts at this station were, in fact, similar to those from station M1, though M2 was much farther from the Sewage Treatment

Plant effluent than was station M1. Contributory effects of the Wye River seemed to cause a more uniform level of bacteria throughout the bay. This was in contrast to Penetang Bay where significant pollution sources appeared to be limited to the South Basin.

Bacteriological sampling in Hog Bay was restricted to stations PM2 and PM3. As was seen in Figure 2, these stations reflected low geometric mean counts of a magnitude comparable to those from stations M3 and P4. It appeared on the basis of counts from these four stations that on the south side of the central basin of Georgian Bay, bacterial levels were relatively uniform, in spite of the variation in quantities of wastes introduced into the different adjoining bays.

The Midland and Port McNicoll stations demonstrated a pattern of increase and decline of coliform counts with temperature similar to that seen at stations P2, P3 and P4 in Penetang Bay. Only at station MSTP did large fluctuations, as seen at station P1, occur. It would seem that large bacterial populations such as existed at station PSTP, and to some extent at stations P1 and MSTP, when continually supported by an inflow of nutrients, were buffered to a large extent from both the adverse and stimulatory effects of temperature.

The current OWRC recreational water criteria call for 2,400 coliforms, 100 fecal coliforms and 20 fecal streptococci per 100 mls. On the basis of the geometric means (Figures 1 and 2), only station PSTP was unacceptable by the coliform, fecal coliform and fecal streptococci criteria (G.m.: 5,470, 1,374 and 42 organisms per 100 mls., respectively).

There were occasions, particularly during August, September and October, when individual counts at stations P1, P2 and MSTP exceeded the recommended criteria for fecal coliform and fecal streptococci. These high counts did not occur frequently enough to raise the geometric mean above the acceptable level. The fact that high counts did occur however, is worthy of note. It would be in the interest of public safety to monitor these areas closely during the late summer when the water is still being used for recreational purposes.

2. Isolation Studies:

From the eleven regular biweekly samples, a total of 1,171 cultures were isolated and identified to the genus level. The percentage occurrence of each genus at each station was calculated and presented in Table I.

The predominant genera appeared to be characteristic of individual stations. For example, at station PSTP, Acinetobacter (30.9%), Aeromonas (17.3%) and Enterobacteriaceae (25.9%) were most frequently isolated, whereas the main genera at station P3 were Bacillus (18.4%), Pseudomonas (12.8%) and Enterobacteriaceae (35.8%). The Enterobacteriaceae was the only group consistently isolated at high levels.

Bennett (1969) reported 18.4% of plate count isolates from Lake Ontario as Enterobacteriaceae. The percentages of Enterobacteriaceae isolations from Midland and Port McNicoll stations were at a comparable level. Station MSTP however was an exception. The percentage of Enterobacteriaceae at this station (6.1%) was comparable to the level reported by Bennett (1969) for Lake Superior samples (5.0%). This low level of recovery of Enterobacteriaceae at station MSTP was very unexpected, and as yet, inexplicable, in view of the high coliform counts obtained at this station.

Higher percentages of Enterobacteriaceae were isolated from Penetang Bay. Percentages ranged from 25.9% to 35.8%. This was a much higher recovery rate than that reported by Bennett (1969) for Lake Ontario.

TABLE I (a) - A comparison of the percentage occurrence of bacterial isolates from total plate counts, grouped according to station.

	<u>PSTP</u>	<u>P1</u>	<u>P2</u>	<u>P3</u>	<u>P4</u>
Achromobacter	1.2	2.7	4.5	6.4	5.5
Acinetobacter	30.9	16.1	21.4	7.3	10.0
Aeromonas	17.3	9.8	8.9	1.8	2.7
Alcaligenes	1.2	1.8	3.6	0.9	2.7
Arthrobacter		2.7	3.6	1.8	0.9
Bacillus	3.7	7.1	9.8	18.4	11.8
Chromobacter	-	-	-	-	-
Flavobacterium	3.7	5.4	7.1	9.1	7.3
Kurthia		0.9	0.9		
Micrococcus				0.9	1.8
Pseudomonas	8.6	18.8	9.8	12.8	9.1
Sarcina	-	-	-	-	-
Yeast		1.8	0.9	0.9	7.3
<u>Enterobacteriaceae</u>	25.9	28.6	25.9	35.8	30.0
Enterobacter	4.9	13.4	8.9	14.7	13.6
Citrobacter	1.2	0.9		0.9	1.8
Proteus				1.8	
Escherichia	2.5		0.9	0.9	0.9
Klebsiella	4.9				
Aberrant Types	12.4	14.3	16.1	17.4	13.6
Miscellaneous	3.7	0.9		0.9	2.7
No growth	3.7	3.6	3.6	2.8	8.2
Total (number)	81	112	112	109	110
- 1171 -					

TABLE I (b) - A comparison of the percentage occurrence of bacterial isolates from total plate counts, grouped according to station.

	<u>MSTP</u>	<u>M1</u>	<u>M2</u>	<u>M3</u>	<u>PM2</u>	<u>PM3</u>
Achromobacter	11.0	5.4	3.5	2.6	2.6	
Acinetobacter	32.9	30.4	25.7	25.2	13.9	10.9
Aeromonas	9.8	5.4	1.8	2.6	7.8	3.6
Alcaligenes	1.2	1.8	1.8	0.9	2.6	0.9
Arthrobacter	3.7	1.8	3.5		2.6	2.7
Bacillus	9.8	8.0	10.6	16.5	14.8	16.4
Chromobacter						0.9
Flavobacterium	12.2	10.7	12.4	13.0	15.7	16.4
Kurthia					2.6	0.9
Micrococcus		3.6			0.9	1.8
Pseudomonas	4.9	13.4	8.0	8.7	8.7	13.6
Sarcina			0.9		0.9	1.8
Yeast		0.9	1.8	3.5		
<u>Enterobacteria- ceae</u>	6.1	17.0	21.2	23.5	18.3	22.7
Enterobacter	2.4	10.7	8.0	9.6	9.6	10.0
Citrobacter	1.2				0.9	
Proteus	-	-	-	-	-	-
Escherichia		0.9				
Klebsiella	-	-	-	-	-	-
Aberrant Types	2.4	5.4	13.3	13.9	7.8	12.7
Miscellaneous	1.2	0.9	4.4	0.9	1.8	1.8
No growth	3.7	0.9	4.4	2.6	7.0	5.5
Total (number) - 1171 -	82	112	113	115	115	110

Of the Enterobacteriaceae identified, the most frequently represented genus was Enterobacter. This was similar to Bennett's findings. However, she also frequently isolated Proteus, but only a small number of these organisms were found in the present survey.

A very large number of Enterobacteriaceae cultures were not amenable to classification. These aberrant types could be divided into groups resembling Enterobacter, Citrobacter and Klebsiella. The majority fell into the first of these three groups. A few cultures remained entirely distinct from any presently defined genus. Bennett (1969) did not report the occurrence of these aberrant types in her work.

The Acinetobacter and Bacillus were the only two groups of organisms which appeared to show consistent trends with regard to water quality at the individual stations. In Penetang Bay, Acinetobacter isolations dropped from 30.9% at PSTP to 7.3% and 10.0% at stations P3 and P4. In Midland Bay, the decrease of percentage isolations was more gradual, 32.9% at MSTP to 25.2% at M3. Stations PM2 and PM3 both had lower numbers of Acinetobacter, 13.9% and 10.9% respectively.

Where Acinetobacter tended to decrease as water quality improved, the Bacillus isolations tended to increase. In Penetang Bay, percentage isolations of Bacillus ranged from 3.7% at PSTP to 18.4% and 11.8% at stations P3 and P4. Isolations in Midland Bay increased from 9.8% at MSTP to 16.5% at M3. 14.8% and 16.4% of isolates at stations PM2 and PM3 were Bacillus species.

In order to demonstrate more clearly any association between percentage frequency of isolation and levels of pollution, plate count isolates within each genus were grouped according to the coliform density of the samples from which they were obtained. The resulting distributions for each genus were tested by a Chi-square test of homogeneity. This grouping according to coliform density with class intervals of one log cycle was presented in Table II. A similar grouping and test of significance was carried out according to total plate count density of samples. This treatment of the data was shown in Table III.

From Table II, it was clear that the percentage incidence of a number of genera was affected significantly by changes in coliform density. The Bacillus and Pseudomonas groups demonstrated a steady decrease in frequency as coliform counts increased. These results bore out the suggested

TABLE II - Generic distribution (%) of bacterial isolates
from total plate counts grouped according to
coliform density of the water sample.

GENUS	Coliforms < 10	Coliforms 11-100	Coliforms 101-1000	Coliforms > 1000	X ²	
No growth	11.9	2.4	3.5	2.4	12.43	S.D.**
Achromobacter	3.4	3.6	9.2	1.1	8.27	S.D.*
Acinetobacter	6.2	12.8	25.1	28.8	18.30	S.D.**
Aeromonas	3.4	3.0	8.1	8.3	4.40	N.S.D.
Alcaligenes	4.0	1.2	1.8	1.3	2.45	N.S.D.
Arthro-Coryne	2.3	3.0	1.1	2.1	0.88	N.S.D.
Bacillus	22.0	19.0	7.8	3.2	18.47	S.D.**
Chromobacterium	0.6	-	-	-	-	N.C.
Flavobacterium	9.6	19.6	11.3	2.7	13.42	S.D.**
Kurthia	1.1	0.6	0.4	0.3	-	N.C.
Micrococcus	3.4	0.6	0.7	-	-	N.C.
Pseudomonas	20.3	19.3	5.7	3.2	25.91	S.D.**
Sarcina	-	1.2	-	-	-	N.C.
Yeast	1.7	3.3	1.4	0.3	2.71	N.S.D.
Miscellaneous	5.1	0.3	0.4	2.4	7.25	N.S.D.
<u>Enterobacteriaceae</u>	5.1	10.1	23.7	44.0	43.85	S.D.**
Enterobacter	2.3	3.6	6.4	21.6	28.06	S.D.**
Citrobacter	0.6	-	0.4	1.3	0.88	N.S.D.
Proteus	-	0.6	-	-	-	N.C.
Escherichia	-	0.9	0.4	0.5	0.34	N.S.D.
Klebsiella	-	-	-	1.1	-	N.C.
Aberrant	2.3	5.1	16.7	19.5	19.51	S.D.**
Total Number Isolates	177	336	283	375		

NC = not calculated, NSD = no significant difference, SD* = significant difference (0.05 level)
SD** = significant difference (0.01 level)

inverse relationship between Bacillus isolation frequencies and water quality which was indicated in Table I. Bennett in her work on Lake Ontario was unable to demonstrate by this method, the adverse effects of high coliform densities on the above-mentioned groups.

The Acinetobacter responded to increasing numbers of coliforms in a manner similar to that indicated in Table I. As coliform counts increased, the frequency of Acinetobacter isolations also increased. This result was similar to that reported by Bennett (1969) who suggested that these organisms, in responding to enrichment of the environment in the manner demonstrated, could serve as useful indicators of eutrophication. The present confirmation of Bennett's findings with regard to the Acinetobacter distribution, rendered her suggestion worthy of further study.

The Flavobacterium and Achromobacter groups were also affected by changing coliform concentrations. These two genera demonstrated preferential coliform densities for growth: for Achromobacter, between 101 and 1000 coliforms per 100 mls., and for Flavobacterium, between 11 and 100 coliforms per 100 mls. This preferential density effect was not demonstrated in Bennett's 1969 data.

It was interesting to see that the density of coliforms affected the isolation frequency of organisms incapable of growth on primary isolation media (referred to as "No growth" in Tables I to IV and VI). The percentage of such isolates decreased as the number of coliforms increased. These organisms appeared to be more susceptible to damage when transferred from their natural to a laboratory environment. They were represented less frequently at high coliform levels where a larger percentage of total plate count colonies seemed to be of a less delicate nature.

In her work in 1969, Bennett was unable to demonstrate any significant effect of coliform densities on the frequency of Enterobacteriaceae isolations. In the present survey, an expected trend of Enterobacteriaceae isolations was seen. The percentage occurrence of this group of organisms increased significantly with increasing coliform counts. The genus Enterobacter and the unclassified aberrant types demonstrated significantly higher percentage recoveries as coliform densities increased. The fact that these groups of organisms increased more rapidly than actual coliforms, was to be expected, since coliform enumeration was limited to those members of the Enterobacteriaceae which were lactose fermenters.

In the grouping of isolates according to plate count densities in Table III, a slightly different pattern of frequencies appeared. The Achromobacter, Pseudomonas and Enterobacteriaceae remained at consistent levels regardless of total plate count densities. Although the family Enterobacteriaceae remained steady, the genus Enterobacter and the aberrant types again demonstrated significant differences as total counts changed. The Enterobacter demonstrated a preferential density for growth, between 12,591 and 39,810 organisms per 100 mls. This was in contrast to its pattern of rising frequency as coliform densities increased (Table II). The aberrant Enterobacteriaceae presented a rather peculiar pattern of change with total plate counts. The percentage occurrence of these organisms was high at the extreme ranges of total plate count densities. In the middle range of total counts, lower percentages of aberrant types were recovered. This fluctuating pattern of recovery of these organisms was inexplicable.

The Acinetobacter and Bacillus, when grouped according to plate count density, demonstrated trends of isolation frequency similar to those seen in the coliform density grouping. The Flavobacterium, instead of indicating a preferential density for growth, steadily declined as total

TABLE III - Generic distribution (%) of bacterial isolates
from total plate counts grouped according to
total plate count density (0.5 log. intervals)
of the water sample.

GENUS	T.P.C. (<u>< 3.6</u>)	T.P.C. (<u>3.61-4.10</u>)	T.P.C. (<u>4.11-4.60</u>)	T.P.C. (<u>4.61-5.10</u>)	T.P.C. (<u>>5.1</u>)	χ^2	
No growth	4.9	5.1	3.3	2.3	4.5	1.41	NSD
Achromobacter	4.1	3.6	4.5	6.1	0.9	3.77	NSD
Acinetobacter	5.7	10.2	19.7	36.2	50.0	65.01	SD**
Aeromonas	4.5	7.2	5.6	7.5	3.6	1.99	NSD
Alcaligenes	1.2	3.0	1.5	1.4	0.9	1.66	NSD
Arthro-Coryne	2.0	3.0	2.6	-	2.7	-	NC
Bacillus	21.1	12.9	9.3	6.1	3.6	17.59	SD**
Chromobacterium	-	-	0.4	-	-	-	NC
Flavobacterium	17.9	15.3	5.6	5.6	2.7	19.24	SD**
Kurthia	0.4	0.6	0.7	0.5	-	-	NC
Micrococcus	0.8	0.3	1.5	1.4	-	-	NC
Pseudomonas	9.3	12.6	13.4	9.9	6.4	3.04	NSD
Sarcina	0.8	-	0.4	0.5	-	-	NC
Yeast	4.5	0.3	2.6	-	-	-	NC
Miscellaneous	1.6	1.8	0.7	3.3	0.9	2.48	NSD
<u>Enterobacteriaceae</u>	21.1	24.0	28.3	19.2	23.6	2.04	NSD
Enterobacter	5.3	6.9	20.4	8.9	4.5	18.27	SD**
Citrobacter	-	0.3	2.2	-	-	-	NC
Proteus	0.4	-	0.4	-	-	-	NC
Escherichia	0.8	0.6	-	0.9	-	0.52	NSD
Klebsiella	-	-	0.7	0.5	0.9	0.86	NSD
Aberrant	14.6	16.2	4.5	8.5	19.1	11.86	SD*
Total Number Isolates	246	333	269	213	110		

NC = not calculated, NSD = no significant difference, SD* = significant difference
SD** = significant difference (0.01 level) (0.05 level)

plate count density increased. This decreasing trend, along with that of the Bacillus, indicated that these organisms either were incapable of competing with large populations of other organisms, or were adversely affected by high nutrient levels.

In order to determine if there was any association between the percentage frequency of isolation and water temperature, plate count isolates were grouped according to "in situ" water temperature of the sample from which they were obtained. This arrangement of the data was presented in Table IV. A Chi-square test of homogeneity was again carried out to determine significant differences between percentage isolations in each class. Because of the large range of temperatures recorded during the survey, temperatures could be divided into only two classes, the first containing temperatures less than 17.5°C, and the second, temperatures greater than or equal to 17.5°C.

Only three genera, Acinetobacter, Pseudomonas and Enterobacter, showed significant differences in percentage of isolations within the two temperature classes. The Acinetobacter were recovered more frequently at higher temperatures, and the Pseudomonas more frequently at lower temperatures. Bennett (1969) demonstrated similar trends

TABLE IV - Generic distribution (%) of bacterial isolates from total plate counts grouped according to "in situ" water temperature of the sample.

GENUS	Temperature	Temperature	χ^2	
	< 17.5	> 17.5		
No growth	6.0	2.1	1.86	NSD
Achromobacter	3.2	5.1	0.43	NSD
Acinetobacter	12.2	27.9	6.13	SD*
Aeromonas	4.5	7.5	0.75	NSD
Alcaligenes	2.5	1.0	0.63	NSD
Arthro-Coryne	2.3	1.9	0.04	NSD
Bacillus	16.1	7.2	3.39	NSD
Chromobacterium	0.2	-	-	NC
Flavobacterium	11.4	9.9	0.11	NSD
Kurthia	0.8	0.2	0.36	NSD
Micrococcus	1.2	0.5	0.28	NSD
Pseudomonas	16.4	4.5	5.55	SD*
Sarcina	0.2	0.5	0.13	NSD
Yeast	2.2	1.0	0.45	NSD
Miscellaneous	3.3	-	-	NC
<u>Enterobacteriaceae</u>	17.6	29.7	3.09	NSD
Enterobacter	5.5	14.3	3.91	SD*
Citrobacter	0.3	0.9	0.30	NSD
Proteus	0.3	-	-	NC
Escherichia	0.3	0.7	0.16	NSD
Klebsiella	0.3	0.3	0	NSD
Aberrant	10.7	13.5	0.28	NSD
Total Number	598	573		

NC = not calculated

NSD = no significant difference

SD* = significant difference (0.05 level)

of Acinetobacter and Pseudomonas isolations, although the temperature range in her data was much lower than that of the present survey.

Bennett (1969) was also able to demonstrate significantly decreasing trends of Flavobacterium and Arthobacter - Corynebacterium isolations. She reported an increasing frequency of Aeromonas isolations. It was not possible to demonstrate the effect of temperature on these genera in the present study.

Although in our study there was no significant difference in Enterobacteriaceae isolations, the genus Enterobacter was more frequently isolated at higher temperatures. Bennett (1969) also reported no difference in frequencies of Enterobacteriaceae in the various temperature ranges. However, she was not able to demonstrate any effect of temperature on the frequency of Enterobacter isolations.

3. Intensive Survey Results:

Bennett (1969 - (b) suggested that one could sample a single season representatively by means of a one week intensive survey (daily sampling) during that season. In order to determine the validity of this suggestion, a ten day intensive survey was carried out from July 14 to 24.

This intensive survey would be representative of the summer months (July to September, inclusive). The geometric means for coliform, fecal coliform, fecal streptococci, Clostridia and total plate counts were calculated for each station for the intensive survey. These means were compared (Table V) with those calculated for the same parameters and stations, using only data collected during July, August and September. The percentage of plate count isolates within each genus were then compared for the same periods of time (Table VI).

From Table V, it was clear that in many cases there were no appreciable differences between the geometric means calculated for the summer months, and those calculated for the intensive survey. There were, however, large differences in the coliform means. In the case of stations P2, P3 and P4, and all the Midland and Port McNicoll stations, the intensive survey geometric means were high enough to render the water unacceptable on the basis of the current criteria of 1000 coliforms per 100 mls. ($\log 1000 = 3.00$) (OWRC, 1970). These same stations demonstrated acceptable mean counts on the basis of the summer data. A second important difference occurred with the fecal coliform means for station PSTP. The summer data mean was at an unacceptable level, while the intensive survey mean was below the standard of 100 organisms per

TABLE V - A comparison of the log. geometric means
of summer and intensive survey data.

<u>Station</u>	<u>Coliform</u>		<u>T.P.C.</u>		<u>Fecal Coli</u>		<u>Fecal Strep</u>		<u>Clostridia</u>	
	<u>Sum.</u>	<u>Inten.</u>	<u>Sum.</u>	<u>Inten.</u>	<u>Sum.</u>	<u>Inten.</u>	<u>Sum.</u>	<u>Inten.</u>	<u>Sum.</u>	<u>Inten.</u>
PSTP	3.87	3.66	5.06	4.61	2.90	1.74	1.59	0.96	1.61	1.76
P1	3.04	3.30	4.35	3.76	1.80	0.94	0.55	0.21	1.18	0.93
P2	2.77	3.56	3.93	4.04	1.19	1.10	0.21	0.18	0.83	0.97
P3	2.42	3.27	4.62	4.09	0.70	0.36	-	0.05	0.73	0.75
P4	2.56	3.61	3.82	4.17	0.79	0.10	-	0.05	0.39	0.26
MSTP	2.15	3.59	4.23	4.53	1.30	0.78	1.39	0.27	0.72	0.85
M1	2.23	3.59	3.91	4.25	0.80	0.07	0.41	0.10	1.11	0.60
M2	2.45	3.83	4.28	4.59	0.39	0.14	0.12	-	0.93	0.78
M3	2.54	3.68	4.12	4.38	0.49	0.05	0.18	-	1.10	0.24
PM2	2.30	3.38	4.01	4.06	0.36	0.19	0.21	0.12	1.16	0.80
PM3	2.19	3.57	3.64	4.17	0.33	-	-	-	0.58	0.37

100 mls. ($\log 100 = 2.0$) (OWRC, 1970). A similar discrepancy occurred for fecal streptococci means for stations PSTP and MSTP. In both cases the summer data means were unacceptable whereas the intensive survey means were less than the recommended 20 organisms per 100 mls. ($\log 20 = 1.16$) (OWRC, 1970). Although there were no further examples of contradictory means with regard to acceptable counts, and although the remaining pairs of means were in most cases equivalent, the fact that the discrepancies noted above, did occur, indicated that the intensive survey was not truly representative of the summer season.

In Table VI, which compared the percentage of plate count isolates within each genus for the summer months and the intensive survey, a very clear distinction arose between the two sets of data. During the intensive survey, very high percentages of Enterobacteriaceae were recovered. At some stations (e.g. MSTP and M3), the percentage occurrence of these organisms was five times as great as their incidence in the regular summer samples. The reverse situation occurred with Acinetobacter isolations. Isolations of this organism were much more frequent during summer sampling than during intensive sampling, for example, at station P4, 29.0% of isolates were Acinetobacter during summer sampling,

TABLE VI - A comparison of the percentage occurrence of
(a) bacterial isolates from total plate counts
from summer and intensive survey sampling.

GENUS	PSTP		P1		P2		P3		P4	
	Sum.	Inten.	Sum.	Inten.	Sum.	Inten.	Sum.	Inten.	Sum.	Inten.
No growth		3.0	5.0	1.0	7.0	3.0	3.0	2.0	3.0	1.0
Achromobacter		7.0		11.0	10.0	10.0	9.0	10.0	13.0	5.0
Acinetobacter	34.0	14.0	23.0	13.0	21.0	4.0	18.0	1.0	29.0	1.0
Aeromonas	22.0	8.0	13.0		13.0	3.0	3.0	2.0	3.0	2.0
Alcaligenes	2.0	1.0			5.0			2.0		
Arthro-Coryne		1.0	5.0		2.0	4.0				
Bacillus	5.0	3.0	5.0	5.0	10.0		15.0	4.0	3.0	
Chromobacterium				1.0						
Flavobacterium	2.0	2.0	3.0	6.0	5.0	3.0	15.0	3.0	11.0	6.0
Kurthia		2.0		2.0		2.0				1.0
Micrococcus		2.0					3.0	1.0		
Pseudomonas	10.0	3.0	15.0	2.0		2.0	6.0	1.0		1.0
Sarcina										1.0
Yeast		2.0	3.0	2.0	2.0			6.0	8.0	
Enterobacteriaceae	25.0	48.0	30.0	56.0	26.0	70.0	29.0	68.0	32.0	82.0
Miscellaneous		1.0		1.0						
TOTAL:	41	90	40	100	42	100	34	100	38	100

TABLE VI - A comparison of the percentage occurrence of
(b) bacterial isolates from total plate counts
from summer and intensive survey sampling.

GENUS	MSTP		M1		M2		M3		PM2		PM3	
	Sum.	Int.	Sum.	Int.	Sum.	Int.	Sum.	Int.	Sum.	Int.	Sum.	Int.
No growth	2.0	6.0		6.0	2.0	4.0	2.0	6.0		2.0		3.0
Achromobacter	19.0	20.0	5.0	5.0	5.0	2.0	5.0	5.0	5.0	2.0		1.0
Acinetobacter	33.0	20.0	41.0	9.0	34.0	10.0	54.8	2.0	26.2	2.0	27.5	3.0
Aeromonas	10.0	7.0	7.0	4.0	2.0	8.0	2.0	9.0	7.1	9.0	5.0	8.0
Alcaligenes	2.0				2.0					1.0		
Arthrobacter	2.0	1.0	5.0		2.0				2.0			
Bacillus	7.0			3.0	10.0	1.0	2.0		19.0	1.0	20.0	
Chromobacterium								1.0				
Flavobacterium	14.0	2.0	7.0	3.0	20.0	6.0	19.0	9.0	23.8	2.0	15.0	1.0
Kurthia		2.0		1.0				1.0	2.0			
Micrococcus		1.0						2.0	2.0	1.0	2.5	1.0
Pseudomonas	2.0	2.0	7.0			1.0	2.0		2.0		7.5	1.0
Sarcina					2.0				2.0		2.5	1.0
Yeast		2.0		1.0								
Enterobacteriaceae	7.0	36.0	27.0	68.0	20.0	69.0	11.9	64.0	7.1	80.0	20.0	81.0
Miscellaneous								1.0				
TOTAL:	42	95	41	100	41	102	42	100	42	100	40	100

during the intensive survey, only 1% of isolates were Acinetobacter. The Enterobacteriaceae and Acinetobacter represented the most consistent differences between the summer and intensive data, in that these differences occurred at all stations. Other incidents of large differences in percentage frequency occurred for individual genera at particular stations, e.g.: Aeromonas at station P1, Bacillus at stations PM2 and PM3, Flavobacterium at station PM2 and Pseudomonas at station P1. From these results it was clear that, as regards percentage recovery of plate count isolates, the intensive survey was definitely not representative of the summer season.

4. Experimental Analyses:

As was mentioned in the section entitled Methods, two experimental membrane filtration enumeration methods were employed. The first of these, for Acinetobacter, was tested by picking characteristic colonies and subjecting them to identification tests as described for total plate count isolates. A total of thirty-four colonies were picked from these Acinetobacter plates. Of these, twenty-two were successfully identified as Acinetobacter. Of the remaining colonies, eight were Aeromonas, two Alcaligenes, one Enterobacteriaceae and one Achromobacter. Although these results indicated some ambiguity as to the reliability of the media in distinguishing

Acinetobacter from other organisms, the results were promising enough to suggest a more extensive testing program. Such a program was planned for 1970.

From the experimental plates for fluorescent pseudomonads, a total of only twenty colonies were picked. These were all identified as members of the genus Pseudomonas. It was planned to subject this media to more extensive testing.

The results indicated that both the experimental enumeration methods may be reliable. The former method, for the detection of Acinetobacter, may prove extremely useful since these organisms appeared indicative of enriched or eutrophic waters.

SUMMARY AND RECOMMENDATIONS

1.) The Sewage Treatment Plant in Penetang Bay contributed significantly to the pollution present in the South Basin. This situation was largely due to the occurrence of bypass in the plant. It is recommended that more detailed records of bypass be kept, including exact times of bypass and estimated volume of flow.

2.) In comparison with the results of the 1967 Penetang survey, water in the South Basin, in the area of stations P1 and P2, had deteriorated. It is recommended that plans to increase the capacity of the Penetang Water Pollution Control Plant be considered, so as to avoid the necessity for bypass. During the late summer, the South Basin should be monitored since high counts of fecal coliform and fecal streptococci occurred during this time.

3.) As was seen in the 1966 survey, unacceptably high counts occurred only at the Water Pollution Control Plant outflow in Midland. Even with the occurrence of occasional high counts, the geometric means for station MSTP were acceptable. Water in the body of the bay itself demonstrated acceptable levels of bacteria, as did stations P3 and P4 in Penetang Bay, and stations PM2 and PM3 in Hog Bay. Stations PM2 and PM3 were considered the cleanest.

4.) Pseudomonas isolations were more frequent at lower temperatures and in cleaner waters (based on coliform density). The Acinetobacter and Enterobacter preferred higher temperatures and more polluted waters. The Bacillus species were associated with unpolluted water, whereas the reverse was true for the Enterobacteriaceae group and the aberrant forms thereof. Both the Flavobacterium and the Achromobacter preferred water of intermediate quality.

5. Bennett's finding (1969) that the Acinetobacter were significantly associated with the level of pollution (as measured by coliform density) was confirmed. These organisms may definitely be useful as indicators of advancing eutrophication.

6.) The results of the intensive survey indicated that at least in the present survey, an intensive survey is not truly representative of the season within which it is carried out.

7.) Experimental enumeration methods for Acinetobacter and fluorescent pseudomonads were sufficiently successfully to justify more extensive testing of them.

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